CHEMOTHERAPY OF MURINE LEPROSY
I. THE USE OF MOUSE LEPROSY AS THE CHEMOTHERAPEUTIC TEST

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INTRODUCTION

Experimental chemotherapy of murine leprosy in both the rat and the mouse has recently been studied by several investigators. The infection was usually produced by subcutaneous inoculation, which results in a disease of very chronic course. By this route the mouse was found to be less susceptible than the rat (2, 5). In both species a long period of observation —i.e., 5 to 18 months—was needed (1, 2, 3). Under these conditions the experimental method leaves much to be desired. Domagk (4) states that "rat leprosy is no test for finding substances useful in the treatment of leprosy."

Mauri and Hadler (11, 12) reported good results by using intraperitoneally infected rats for the study of antileprosy compounds. By this technique they showed definite antileprosy activity of compounds tested. The main disadvantage was that up to 18 months was required to finish the experiment.

Grunberg and Schnitzer (6) reported a chemotherapeutic study of 200 compounds in intraperitoneally-infected mice. Smears were made from small lesions removed from the abdominal cavity. The bacillus count was used as the only criterion for drug activity. An advantage of this method is that the observation period was shortened to 28 days. However, only one or two out of the 200 compounds tested were found to possess activity, even though several of them are effective in human leprosy. This method, therefore, does not appear to be reliable for this purpose.

Using intracerebrally-infected mice, Levaditi and his associates (10) found the antileprosy activity of three compounds—streptomycin, 4,4'-diaminodiphenyl sulfone and para-aminosalicylic acid—to be approximately similar to that exhibited in clinical cases of leprosy. The experimental period was 77 days.

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Histological sections of the brain were made. The bacillus counts and the meningeal lesions were used as the criteria for the evaluation of drug activity. This method dealt chiefly with a histological technique, which is tedious for routine chemotherapeutic assay.

From this brief review of the literature it is obvious that a simple and reliable method for the study of the chemotherapy of murine leprosy is desirable. The experimental period should be reasonably short, and the evidence of antimicrobic activity should be clear-cut and reproducible.

Since November 1950, we have studied the possibility of using the intraperitoneally-infected mouse as the chemotherapeutic test object. In white mice infected by this route the disease has a more rapid course than in rats. The internal organs reveal marked leprous growth in the third and the fourth months after inoculation. Antileprosy activity of drugs can be clearly shown within three months, and their effectiveness can be measured by the gross pathological lesions. The results have been found to be reproducible, and large numbers of animals can be used to make the experiments statistically significant. Four series of chemotherapeutic experiments have been performed, employing a variety of antileprosy agents. A total of 872 mice have been used without a single animal failing to develop macroscopic leprous lesions, apart from a few animals that were found dead and partially destroyed by other mice.

The present report includes: (1) a study of the evolution of intraperitoneally-infected mouse leprosy, and (2) a description of the chemotherapeutic test in intraperitoneally-infected mouse leprosy. The antileprosy activity of different compounds will be reported in later communications.

METHODS

The animals used were young white female mice of the general-purpose strain of the National Institutes of Health, fed with ground Purina Rat Chow and weighing 20 ± 2 gm. They were used in groups of 20. The Hawaiian strain of rat leprosy was used.[2] The original inoculation material was a suspension of a subcutaneous leproma of a rat; this was inoculated in 0.5 ml. doses intraperitoneally into 20 mice. The evolution of the infection was studied by killing 3 mice every month. It was found that the omentum and the pelvic fatty pads were tremendously enlarged in the later stages of the disease. They were studded with lepromatous growth, which could be used as the transmitting material for inoculating a large number of mice.

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[2] Obtained from Dr. J. H. Hanks, bacteriologist, Leonard Wood Memorial, Harvard Medical School, Boston.

Since then, four serial transmissions for chemotherapeutic studies have been performed by inoculating such material into a total of 852 mice using, successively, suspensions from mice of the preceding generation. For these inoculations material was obtained from mice killed 4-5 months after inoculation. The omenta and pelvic fatty pads were removed and ground with fine sand in a mortar. Sodium chloride solution (0.9%) was added to make the dilution approximately 1:30, and the suspension was filtered through four layers of gauze. The animals were injected under aseptic conditions with 0.5 ml. of the filtered suspension intraperitoneally, using a 5 ml. syringe and 23 gauge needle.

A numerical recording, designated hereafter as the "leprosy index," was used to record the extent of leprous involvement in the different organs. The grading was from 0, for no visible lesions, to 6, for the most extensive lesions as found in the peritoneal cavity. For the different sites and organs the following grades were used: (*a*) site of inoculation, 0-2; (*b*) omentum and peritoneum (chiefly the mesentery), 0-6; (*c*) pelvic fatty pads, 0-4; (*d*) lymph nodes, including portal, paravertebral (above the bifurcation of iliac arteries) and tracheobronchial groups, 0-2; (*e*) spleen, 0-4 (since enlargement of the spleen is frequently found in normal mice, the leprosy index was recorded only in cases of marked enlargement or when gross leprous lesions were found); (*f*) liver, 0-4; (*g*) miscellaneous; lungs, kidneys, diaphragm, pericardium, retrosternal region, and thymus, each of six, 0-1.

The upper limit of the leprosy index of the whole animal is 28. This is the chief criterion for evaluating the antileprosy activity of drugs. The other criteria may be used as supplementary aids.

In order to prepare smears, tissues were first minced with a scalpel. The slides were fixed at 100°C. for five minutes. Kinyoun's modified acid-fast stain ([8]) was used,[3] staining the smear for 3 minutes (no heat necessary), and further steps were continued as with the Ziehl-Neelsen stain. Hanks' method ([7]) for recording the bacterial counts (bacillus index) was found convenient, the number of bacilli being graded as of 0 to 5 values.

Histological sections were made in a few cases in the preliminary study, and in some instances in the chemotherapeutic investigation.

EVOLUTION OF MOUSE LEPROSY FOLLOWING INTRAPERITONEAL INOCULATION

Table 1 shows the course of evolution of the lesions of intraperitoneally-infected mice. They represent three series of observations made in parallel with three successive chemotherapeutic experiments. Twenty mice were included in each series, and three were killed every month. The lesions and the numbers of acid-fast bacilli increased steadily with the duration of the infection. Deaths from extensive leprous involvement began at the end of the third month. No animal survived beyond six months. The mortality rate could not be determined in this ex-

[3] Basic fuchsin, 4 gm.; phenol crystals, 8 gm.; alcohol, 95 per cent, 20 ml.; and distilled water, 100 ml.

periment, as the majority of animals were killed before six months.

TABLE 1.—*Evolution of murine leprosy in the intraperitoneally-infected mouse; the leprosy index.*

Duration, months	Serial number	Body weight, gm.	Leprosy index								Weight of Lepromatous tissues, gm.	
			Site of inoculation	Omentum & peritoneum	Pelvic fatty pads	Lymph nodes	Spleen	Liver	Miscellaneous	Total index	Omentum	Pelvic fatty pads
		22	0	1	0	0	0	0	0	1.0		
	1	25	0	1	0	0	0	0	0	1.0		
		26	0	1	0	0	0	0	0	1.0		
		20	0	1	0	0	0	0	0	1.0	0.11	0.43
1	2	20	0	1	0	0	0	0	0	1.0	0.06	0.11
		20	0	+	0	0	0	0	0	0.5	0.08	0.57
		30	+	++	0	1	0	0	0	2.0	0.03	0.16
	3	24	0	++	0	1	0	0	0	1.5	0.03	0.16
		26	0	+	0	1	0	0	0	1.5	0.04	0.21
		25	0	1+	1	0	0	0	0	2.5		
	1	28	0	2	0	1	0	+	0	3.5		
		26	1	2	0	0	0	0	0	3.0		
		24	0	1	0	1	0	0	0	2.0	0.09	0.62
2	2	28	0	2	0	0	0	1	0	3.0	0.15	0.79
		24	1	1	1	1	0	0	0	4.0	0.06	1.24
		28	2	2	0	1	0	0	0	5.0	0.08	0.60
	3	28	1	1	0	0	0	0	0	2.0	0.02	0.51
		22	2	2	1	0	0	0	0	5.0	0.09	0.50
		26	2	4	3	1	1	2	0	13.0		
	1	28	2	3	3	1	0	0	0	9.0		
		22	2	4	4	1	0	0	0	11.0		
		22	2	3	3	0	0	0	0	8.0	0.10	1.43
3	2	25	2	4	3	1	0	2	1	13.0	0.15	1.31
		21	1	4	4	1	2	2	0	14.0	0.23	1.41
		25	1	3	2	2	0	1	0	9.0	0.15	0.98
	3	23	1	6	4	2	1	0	0	14.0	0.35	1.46
		35	1	3	1	1	0	0	0	6.0	0.15	1.78
		25	1	6	4	2	1	0	1	15.0		
	1	22	1	6	4	2	1	3	2	19.0		
		25	+	6	4	2	1	0	1	14.5		
		17	1	3	3	1	1	4	2	15.0	0.06	0.72
4	2	27	2	4	3	1	4	1	2	17.0	0.23	1.19
		30	2	5	2	2	0	2	1	14.0	0.30	0.94
		32	+	6	4	1	3	3	1	18.5	0.25	2.64
	3	16	+	6	2	1	1	2	1	13.5	0.24	0.54
		28	1	6	2	2	2	4	1	18.0	0.21	0.81

A detailed description of the progress of the leprous growth in individual organs follows:

Site of inoculation.—A small nodular lesion usually developed in the subcutaneous tissue around the point of injection. The adjacent fatty tissue was frequently involved. Caseation was the rule, with occasional ulceration. Acid-fast bacilli were abundant in the caseous material, and they were often present in places where no gross lesions were visible. Abdominal

muscles were frequently involved. Enlargement of inguinal lymph nodes was not uncommon.

Omentum.—The omentum was constantly involved. After one month of infection scattered tiny nodules were seen, and by the third month large lepromata were frequent (Fig. 1). Later, the growth became very large. Caseous material was frequently present in the centers of the large lepromata. Smears showed acid-fast bacilli which increased in amount with the duration of infection. The histological structure is illustrated in Fig. 2.

Pelvic fatty pads.—The involvement of the pelvic fatty pads was the most characteristic lesion at the third month. In Fig. 1 the large, lobulated fatty pads can be seen below the uterine horns. These consisted mainly of the leprous growth infiltrated into the fatty tissue normally present. Fig. 3 shows the histological appearance. The typical lepra cells are crowded with bacilli.

Mice normally possess a certain amount of fatty tissue in the pelvic region, probably in the broad ligment in the female, which increases gradually with age. The leprous growth invaded the fatty tissue in the first month of the infection, and the amount gradually increased to a peak in the third and fourth months. Adhesions were rare. Smears showed various amounts of acid-fast bacilli. Variation in the extent of leprous involvement of pelvic fatty pads was great. Some animals developed only slight lesions after three months, while others had extremely large growths. The amount of pelvic fat in normal mice also varies markedly. In Fig. 4 the fatty pads and omenta of 19 normal mice are contrasted with those of 17 untreated leprous mice killed three months after inoculation.

Peritoneum.—Numerous tiny nodules over the mesentery were common. Extensive leprous growth occurred in the later stages. Large nodules over the mesentery occasionally occurred. A single nodule was frequently found around the gall bladder. Extensive lesions of the diaphragm were frequent, and leprous growth in the retrosternal region developed in severe cases. Ascites was a rare complication (as was pleural effusion).

Lymph nodes and thymus.—The retroperitoneal lymph nodes were infected as a rule. The portal, mesenteric, paravertebral and tracheobronchial nodes were frequently found enlarged after the third month, at times reaching tremendous size. The number of bacilli found in smears was variable. The thymus gland was frequently enlarged.

Spleen.—Acid-fast bacilli were found in smears of the spleen after one month. Splenic enlargement with miliary leprous lesions throughout the organ was seen in the later stages. Histological examinations showed numerous lepra cells scattered in groups through the sections.

Liver.—The liver frequently showed scattered lesions in the third month, more severe involvement occurring later. At times the whole organ was covered with pale miliary lesions. Acid-fast bacilli were demonstrable in the first month and later increased in numbers. In the fourth and fifth months the whole liver was studded with lepra cells, and, as in the spleen, smears revealed swarms of bacilli. Survival did not seem possible with this degree of bacterial invasion.

Lungs.—Congested areas were frequently seen in the lungs. Smears were made in all instances, but acid-fast bacilli were seldom observed. Histological sections made in three cases showed leprosy bacilli in one.

Kidneys.—As a rule there were no gross pathological lesions in this organ. In histological sections lepra cells were occasionally observed in the capsule.

Heart.—Acid-fast bacilli were found in histological sections of the heart in the later stages. The pericardium was frequently involved.

Gastro-intestinal and genito-urinary tracts.—No gross pathological lesions were seen over the stomach, intestine, uterus or urinary bladder. Acid-fast bacilli were found in sections of the small intestine and uterus.

General condition.—The general condition and the body weights of the animals usually remained good. When death occurred in the early stages it was generally the result of intercurrent infection. When the disease approached its terminal stage, with severe involvement of vital organs, it was accompanied by malnutrition, depression and weakness, ending in the death of the animal.

Evolution of the infection in the male mouse, studied in only ten animals, was found to be similar to that in the female.

CHEMOTHERAPEUTIC TEST IN INTRAPERITONEALLY-INFECTED MOUSE LEPROSY

In the study of the antileprosy activity of drugs in mouse leprosy the duration of the experiment is very important. It is essential that the period of observation be sufficient to per-

mit lepromata to develop to a stage where they are readily detectable macroscopically.

From Table 1 it is seen that at the end of the first or second month after the inoculation, the gross lesions were minimal. After three months, however, the lesions in various organs became so prominent that they could not escape observation. The microorganisms also became more numerous. (Table 2.)

TABLE 2.—*Evolution of murine leprosy in the intraperitoneally-infected mouse; the bacillus index.*

Duration, months	Serial number	Bacillus index										
		Site of inoculation	Omentum	Pelvic fatty pads	Portal lymph nodes	Paravertebral lymph nodes	Bronchotracheal nodes	Spleen	Liver	Lung	Kidney	Total index
1	1	0	3	2	0	0	2	0	0	0	0	7
		0	3	0	0	0	1	1	1	0	0	6
		0	2	3	0	1	1	0	1	0	0	8
	2	0	2	2	0	0	2	0	1	0	0	7
		1	2	0	0	0	1	0	1	0	0	5
		0	0	1	1	1	1	0	1	0	0	5
	3	3	3	1	1	0	2	0	0	0	0	10
		0	2	1	1	0	2	0	0	0	0	6
		2	0	1	0	0	1	0	0	0	0	4
2	1	3	4	1	1	1	0	0	1	0	0	11
		3	4	4	1	0	1	0	1	0	0	14
		4	4	4	2	1	1	0	0	0	0	16
	2	1	2	1	1	1	2	1	1	0	0	10
		1	1	1	1	0	2	0	1	0	0	7
		2	2	1	1	1	1	0	0	0	0	8
	3	2	3	1	0	0	1	1	0	0	0	8
		2	3	1	1	1	1	1	0	0	0	10
		2	4	2	1	0	2	1	2	0	0	14
3	1	5	4	4	1	1	3	1	1	0	0	20
		5	5	3	0	1	2	0	1	0	0	16
		5	4	4	1	1	3	1	1	0	0	20
	2	4	3	5	2	2	2	2	2	0	0	22
		5	3	4	1	3	3	2	2	0	0	23
		4	4	2	2	2	2	2	2	0	0	20
	3	4	2	3	0	1	0	1	1	0	0	12
		3	4	2	0	2	2	1	2	0	0	16
		4	2	2	1	1	1	1	2	0	0	14
4	1	5	5	5	3	2	3	2	1	0	0	26
		5	5	5	3	2	4	2	3	0	0	29
		5	5	5	2	2	3	2	1	0	0	25
	2	3	5	4	1	1	3	4	1	1	0	23
		4	4	4	4	3	4	3	3	1	0	30
		4	3	3	2	3	3	3	4	0	0	25
	3	4	5	5	3	3	3	3	3	1	1	31
		4	4	3	3	3	3	4	3	2	0	29
		3	5	4	2	3	3	2	4	2	0	28

By using the three-month period for the chemotherapeutic test a sharp contrast was seen between the leprous lesions of effectively treated and untreated animals. During the past

one-and-one-half years we have tested various antileprosy drugs in 740 leprous mice in four series of experiments; clear-cut differences were seen in gross pathological lesions, in bacillus counts, and in histological sections. The findings will be reported in detail in later communications.

Treatment in the therapy groups was started on the day after inoculation. Drugs were given mixed in the diet or by injection. The average daily food consumption was 3 to 4 grams. Animals that died in the first two weeks were replaced from a spare group. Any animal found dead after two weeks was autopsied as soon as possible. At the end of three months all the animals were sacrificed. The body weight and the leprosy index of each animal were recorded. The omentum and pelvic fatty pads were removed, weighed, and photographed in groups. Smears of tissues from two representative individuals of each group were made as follows: site of inoculation, omentum, pelvic fatty pads, lymph nodes (portal, paravertebral and tracheobronchial), spleen, liver and lungs, and the bacillus index was recorded.

The following groups of 20 mice each were employed: one group of untreated animals was used as the leprosy control; one group was treated with either streptomycin or sulfone as the standard of reference; one or two groups were treated with each dose of the drug; and one group of uninfected animals was treated with the drug as the toxicity control. Several drugs were tested in each experiment.

The methods of arriving at the leprosy index and the bacillus index have been described. In the experimental work, an index of chemotherapeutic effectiveness (I.C.E.) is calculated as follows:

$$\frac{\text{Total leprosy index of the control group}}{\text{Total leprosy index of the treated group}}$$

This index may be used in the comparison of the antileprosy activity of different drugs. The larger the figure, the higher is the activity. Unity means no action.

DISCUSSION

The pathological changes of mouse leprosy have been studied by several investigators. Sellards and Pinkerton (13) reported the pathological changes in mice infected with the Stefansky bacillus by the subcutaneous, intraperitoneal, intracerebral, and other routes. Krakower and Gonzales (9) described lesions produced by subcutaneous, intramuscular and intraperitoneal inoculation of tissue from a naturally infected wild mouse. They considered that their observations agreed essentially with the findings of Fite (5), who reported on the pathological findings in rat and mouse leprosy after subcutaneous inoculation. These findings also agree with ours on lesions in intraperitoneally-infected mouse leprosy.

Sellards and Pinkerton (13) reported that pure strains of mice (dilute brown, C-57 black, and Swiss strain) were more susceptible to the subcutaneous infection than mixed breeds. De Souza-Araujo (14) found that the C-57 black mouse was more susceptible to the subcutaneous infection than any other animal used. It is, therefore, reasonable to suppose that some mouse strain can also be found in which a much more rapid course of infection will occur than in the strain we have employed. Also strains of *M. leprae murium* more virulent than the Hawaiian may be found.

SUMMARY

The evolution of intraperitoneally-infected mouse leprosy is described.

A procedure of chemotherapeutic assay based on intraperitoneal inoculation of mice is outlined.

RESÚMEN

En éste artículo, el cual es introductorio a otros reportes sobre la quimoterapia de la lepra murina en el ratón inoculado por vía intraperitoneal, se describen los métodos empleados y las lesiones que se desarrollaron en los varios órganos. Se usó el ratoncillo blanco cepa "National Institutes of Health" y se utilizaron solo hembras de 20±2 gramos de peso en grupos de 20 para compensar las variaciones individuales y que los resultados tuvieran valor estatístico. Se usó la vía intraperitoneal porque así se producen marcadas lesions en menos tiempo que por vía subcutánea. Se comenzó el tratamiento al dá siguiente de las inoculaciones y el período de observacion fué de 3 meses. Se describe el método de evaluar las lesiones por medio del "índice leproso", y el "índice bacilar" se obtieve calibrando el número de bacilos en frates del 0 al 5. El "índice de efectividad quimoterapéutica" se obtiene dividiendo el "índice leproso" de los testigos por el de los animales tratados. Las diferencias entre animales tratados y los testigos son enteramente claras y reproducibles.

REFERENCES

1. Carpenter, C. M., Stokinger, H. E., Suhrland, L. G. and Ackerman, H. Chemotherapy of murine leprosy. American Rev. Tuberc. **60** (1949) 359-365.
2. Chaussinand, R., Paris, C. and Crougue, O. Essais de traitement par la streptomycine de l'infection murine due au bacille de Stéfansky. Ann. Inst. Pasteur **75** (1948) 92-94.
3. Cowdry, E. V. and Ruangsiri, C. Influence of promin, starch and heptaldehyde on experimental leprosy in rats. Arch. Path. **32** (1941) 632-640.
4. Domagk, G. The chemotherapy of tuberculosis with thiosemicarbazones. Colloquium on the chemotherapy of tuberculosis. Medical Research Council of Ireland, Dublin, 1951, p. 136.

5. FITE, G. L. Leprosy; the pathology of experimental rat leprosy. Natl. Inst. Hlth. Bull. No. 173, 1940, pp. 45-76.
6. GRUNBERG, E. and SCHNITZER, R. J. Chemotherapy of murine leprosy. Ann. New York Acad. Sci. **54** (1951) 107-114.
7. HANKS, J. H. and BACKERMAN, T. The tissue sites most favorable for the development of murine leprosy in rats and mice. Internat. J. Leprosy **18** (1950) 185-207.
8. KINYOUN, J. J. A note on Uhlenhuth's method for sputum examination for tubercle bacilli. American J. Publ. Hlth. **5** (1915) 867-870.
9. KRAKOWER, C. and GONZALES, L. M. Mouse leprosy. Arch. Path. **30** (1940) 308-329.
10. LEVADITI, C. and CHAIGNEAU-ERHARD, H. Activité anti-microbienne de la streptomycine, de l'acide p-aminosalicylique et de la diamino-diphényl-sulfone chez les souris contaminées par le bacille de Stefanski. Compt. rend. Soc. Biol. **145** (1951) 328-330.
11. MAURI, A. C. and HADLER, W. A. Quimioterapia da lepra. Estudos experimentais. Rev. brasileira Leprol. **17** (1949) 140-143.
12. MAURI, A. C., HADLER, W. A. and CARVALHO, C. M. Quimioterapia da lepra. I. Ação do 4,4'-diamino-difenil-sulfona na lepra murina. Rev. brasileira Leprol. **19** (1951) 85-116.
13. SELLARDS, A. W. and PINKERTON, H. The behavior of murine and human leprosy in foreign host. American J. Path. **14** (1938) 421-434.
14. DE SOUZA-ARAUJO. H. C. Rat leprosy; susceptibility of the black mouse (American race) to the Stefansky bacillus; preliminary report. Internat. J. Leprosy **18** (1950) 49-52.

DESCRIPTION OF PLATE

PLATE (1)

FIG. 1. Murine leprosy lesions in the mouse, three months after intraperitoneal inoculation. The following lesions are to be seen: a large leproma over the omentum, between the stomach and spleen; large, lobulated, nodular masses in the pelvic region below the uterine horns, designated the "pelvic fatty pads"; one nodule near the gallbladder; nodular lesions of the mesentery. The thymus gland is enlarged.

FIG. 2. Histological section of a lepromatous lesion in the omentum, three-month mouse leprosy. The large nodule is composed almost entirely of lepra cells. Ziehl-Neelsen stain, 105X.

FIG. 3. Histological section of a lesion in the pelvic fatty pad, three-month mouse leprosy. The large lepra cells are crowded with *Mycobacterium leprae murium*. Ziehl-Neelsen stain, 1200X.

FIG. 4. Pelvic fatty pads (upper) and omenta (lower) of 19 normal mice contrasted with those of 17 untreated leprous mice killed three months after inoculation.

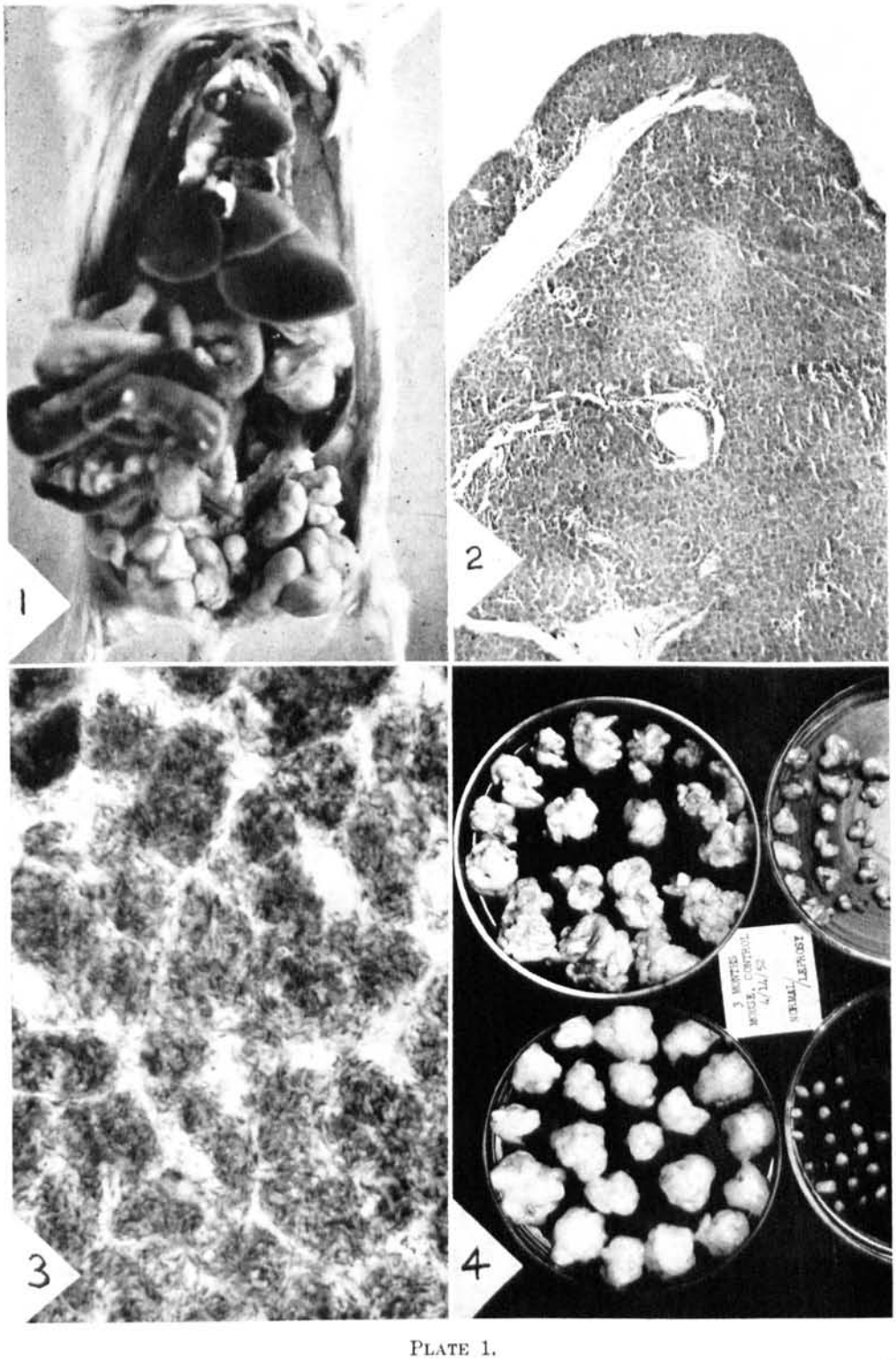

PLATE 1.